BIODEGRADATION AND OXIDATION APPROACHES FOR THE DEMILITARIZATION OF VX HYDROLYSATE

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ABSTRACT

Under U.S. law and the terms of the Chemical Weapons Convention (CWC), the U.S. Army is required to destroy its stockpile of chemical warfare agents (30,000 tons) by April 2007. Public and political opposition to incineration lead to evaluation of several alternative technologies, including biodegradation [1]. These alternatives involved an initial chemical neutralization (hydrolysis) reaction that reduces the toxicity of the agents, followed by a secondary treatment that further degrades and detoxifies the hydrolysis products, some of which are also covered by the CWC (Schedule 2) [2]. Because of the recalcitrant nature of some of the products (as well as the hydrolyzed explosives/propellants that could be present), the use of advanced oxidations processes (AOP) was combined with biodegradation in a variety of configurations to determine whether complete removal and detoxification of the prohibited treaty materials could be achieved. Results using UV/Peroxide and Ozone treatments, prior to or after biodegradation of caustic hydrolyzed VX will be presented. Bioreactors evaluated included sequencing batch reactors (SBRs) and immobilized cell systems.

INTRODUCTION

VX (diisopropylaminoethyl methylphosphonothioate) is the most toxic nerve agent in the U.S. stockpile having an LD_{50} of 0.001 mg/kg (i.v.). Current protocols for the demilitarization of VX involve hot (90ºC) alkaline hydrolysis followed by a secondary treatment of the hydrolysate [2]. The primary constituents of the hydrolysate are ethylmethylphosphonic acid (EMPA) and diisopropylaminoethylthiol (and its disulfide). Looking at the elemental components of the hydrolysate (carbon, nitrogen, phosphorus and sulfur) it would appear to be ideal for biodegradation by microorganisms. However, there are several factors that have previously resulted in poor results [3]. Both phosphorus and sulfur are in large excess when compared to the amount of carbon that is present. The P-C bond of EMPA (and its product, methylphosphonic acid – MPA) is recalcitrant to most organisms and generally only metabolized when there is little or no inorganic phosphate available [4,5]. In addition, the VX-thiol and its disulfide have a significant toxicity as well as a tertiary amine that is also very resistant to microbial attack. While some of these limitations (excess phosphorus and sulfur) can be addressed by the addition of extra carbon sources, this greatly increases the scale and cost of the process and has not been demonstrated to be totally effective. Further complicating the situation is the fact that in some situations it will also be necessary to degrade the alkaline hydrolysate of the explosive Composition B-4 (Comp B). Comp B consists of 59.75% RDX, 39.75% TNT and 0,5% calcium silicate. The exact nature of the hydrolysate products is unknown. It is an intense brown/black color (even when diluted 100-fold) and has considerable environmental toxicity. It was decided that combining biodegradation before or after oxidation could provide a solution to this problem and several approaches were examined.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 JUL 2003	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Biodegradation And Oxidation Approaches For The Demilitarization Of Vx Hydrolysate	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Edgewood Chemical Biological Center Aberdeen Proving Ground, MD 21010-5424**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001523., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**6**	

APPROACHES

Three approaches combining biodegradation with advanced oxidation processes (AOP) are reported on in this paper.

The first approach (Method #1) was a three-step process consisting of biodegradation of VX hydrolysate (HVX), treatment of the bioreactor effluent with UV/peroxide, and then secondary biotreatment with or without the addition of hydrolyzed Comp B. These reactors are shown below:

Bioreactor VX-2a

UV/Peroxide Reactor

Bioreactor VX-2b

Both bioreactors were run as sequencing batch reactors[3]. The working volumes of the cultures were 2.75 liters with a daily draw/feed volume of 230 ml, giving a hydraulic residence time of 12 days. The pH of both systems was maintained at 7.5 through the automatic addition of acid (mixture of 0.5 M each acetic acid, formic acid and HCl). Temperature of operation was room temperature ~25ºC. The SBR cycle consisted of a 16-hour aerated react phase, a 90-minute settle phase, a 30-minute draw phase and a 6-hour aerated feed phase. The feed for Bioreactor #1 consisted of (per liter): HVX (10 ml); NH_4Cl (0.40 g); KCl (0.25 g); and a trace metals solution (5 ml) [6]. The effluent from Bioreactor #1 was clarified by centrifugation and treated with UV/peroxide in a 1-liter quartz reactor. The UV was provided by a medium pressure, high intensity light. Initial reaction time was two hours. This was incrementally extended to four hours as the HVX concentration in the feed to Bioreactor #1 was increased from 1 to 3%. Hydrogen peroxide (30%) was added at the start of the reaction to give a concentration of 10,000 ppm. Aliquots of hydrogen peroxide were added as required to maintain >1000 ppm peroxide. Solid or liquid NaOH was added as necessary to maintain the pH near 7.0. Upon completion of the reaction, the enzyme catalase was added to remove residual peroxide. This effluent was fed directly into Bioreactor #2. Initially, 17 ml/L Comp B hydrolysate was added. However, since the major concern of the study was for the destruction of CWC products, the Comp B hydrolysate was later eliminated.

Method #2 involved the pretreatment of the HVX and Comp B hydrolysates prior to biodegradation. For this approach, the method of AOP selected was ozone treatment. Since mixing the hydrolysates before ozone treatment resulted in excessive foaming, they were 2X solutions were treated separately and then mixed to give the final bioreactor feed. Initial HVX concentration (in the final feed) was initially set at 1% and then gradually increased to 2%. The 2X HVX was treated with ozone for 4 hours. The Comp B hydrolysate was set at 1.7% throughout the study and was ozone treated for 2 hours. This duration of treatment did not result in any significant drop in COD levels, but converted the brown/black solution to a clear one with only a slight yellow or green tint. The ozone treated hydrolysates were combined and supplemented with NH_4Cl (0.40 g); KCl (0.25 g); and a trace metals solution (5 ml). The SBR bioreactor (VX-1) used for this study had a 1.2-liter working volume and a hydraulic residence time of 12 days. The operating conditions of the bioreactor were the same as

in Method #1 (SBR cycle times, pH 7.5, temperature ~25ºC, and automatic addition of the acid mixture – 0.5 M each acetic acid, formic acid and HCl).

The third Method involved the use of an immobilized cell bioreactor and pretreatment oxidation of the HVX. A 5% solution of HVX was treated with ozone for 6 hours and supplemented with NH_4Cl (0.40 g); KCl (0.25 g); and a trace metals solution (5 ml). The starting pH of the feed was 6.0 and the bioreactor operated at room temperature. The bioreactor consisted of 5 x 80 cm column filled with Johns-Manville Ceramic Support. The total liquid volume of the column was ~400 ml. The initial flow rate was set to give an HRT of 5.6 days. Because of the set up of the system, no pH control was feasible.

All bioreactors were inoculated with sludge from the Aberdeen Municipal Waste Treatment Plant and biomass saved from previous cultures aimed at biodegrading HVX.

RESULTS

Method #1: As expected from previous studies [3], the initial bioreactor was able to effectively convert EMPA to MPA. However, degradation of significant quantities of MPA was also observed, even in the presence of relatively high levels (100-200 ppm) of inorganic phosphate. This correlated to the color of the culture gradually turning pinkish in color. The color was caused by growth of the methylotrophic bacterium *Methylobacterium radiotolerans*. Although the mechanism of MPA degradation has not yet been characterized, it is believed that this organism is able to use it as a carbon source. MPA is used by many organisms as a phosphorus source, but the enzyme responsible for its metabolism, C-P Lyase, is strongly repressed by even low levels of phosphate. By using the MPA as a carbon source, presumably a different enzyme system is being used – one that is not repressed by phosphate. Also as previously seen, the organosulfur compounds (VX-thiol and disulfide) are not metabolized to any significant degree. However, they are totally converted to non-sulfur products by the UV/peroxide treatment. Some further degradation of the tertiary amine was also occurring based on the appearance of nitrate in the reactor product. Since the aim of the UV/peroxide treatment was not complete mineralization of the organics, but just conversion of them into more biodegradable products, there was always some MPA incorporated into the feed for the second bioreactor. In spite of the high levels of inorganic phosphate (500-1000 ppm), the residual MPA was removed by the biomass. Based on COD levels, there was not significant degradation of the Comp B hydrolysate components. Presumably, some of the more readily biodegradable materials were removed but what these materials are is unknown at this time. The results of Method #1 at 1% HVX in regards to the destruction of CWC materials is shown in Figure 1.

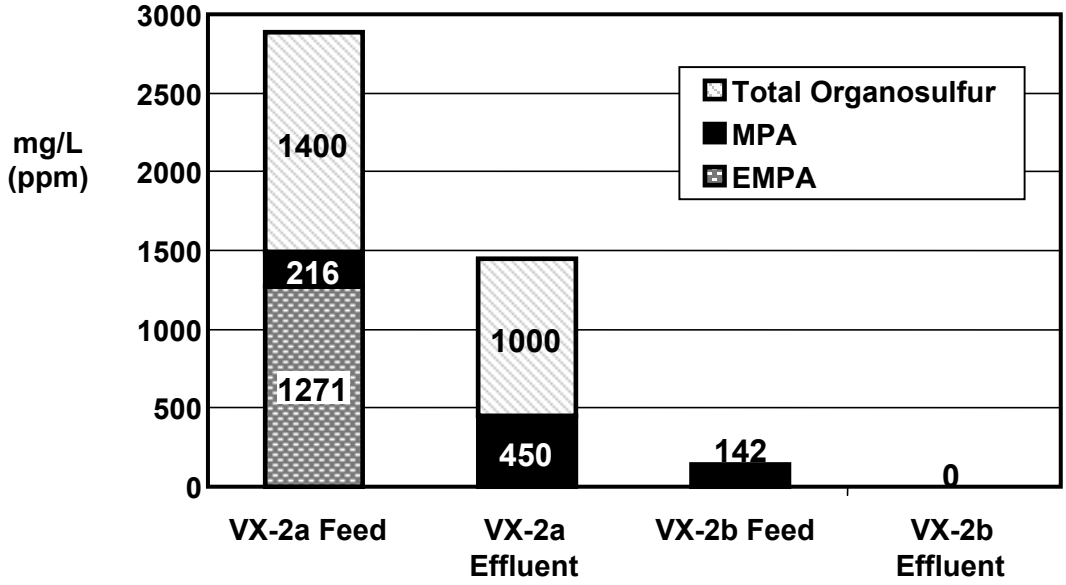

Figure 1. Degradation of CWC compounds by Method #1.

In addition to removing the CWC compounds, the combined treatment system also greatly reduced the toxicity of the HVX and Comp B hydrolysates based on microbial toxicity testing (Microtox®). The final effluent coming from the second bioreactor was completely non-toxic according to this assay. This is illustrated in Figure 2 in which the higher the value, the lower the toxicity.

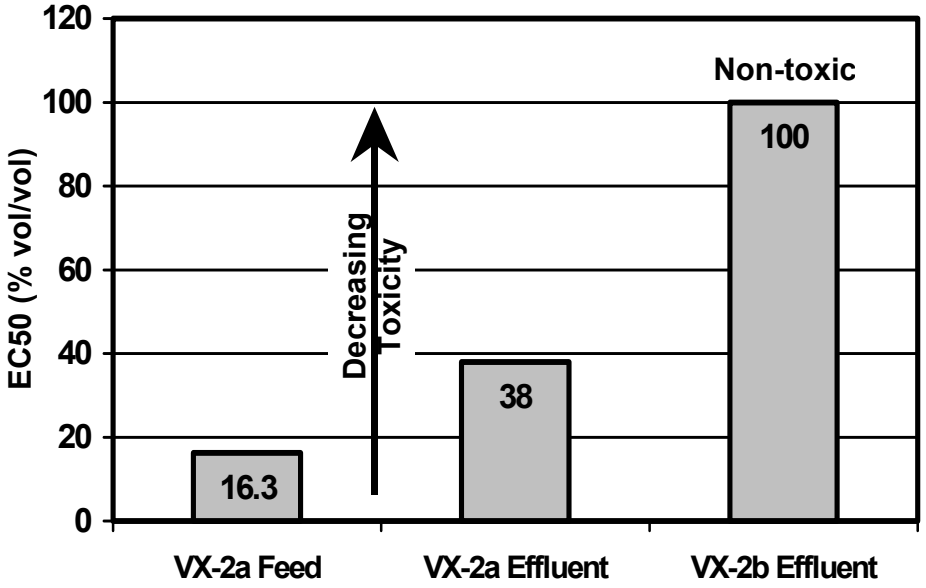

Figure 2. Microtox® results with feed and effluents from Method #1.

As the level of HVX in the initial feed increased, there was a resultant increase for MPA reaching the second bioreactor. Without changing any of the parameters, MPA began to appear in the effluent of the second bioreactor. However, increasing the UV/peroxide treatment time or the hydraulic residence time of the bioreactor may alleviate the situation.

Method #2: The pretreatment of 2% HVX with ozone was effective at eliminating the organosulfur compounds. However, unlike UV/peroxide, did not have as significant an effect on the mineralization of EMPA and MPA. As shown in Figure 3 the bioreactor was could convert most of the EMPA to MPA but not to completion.

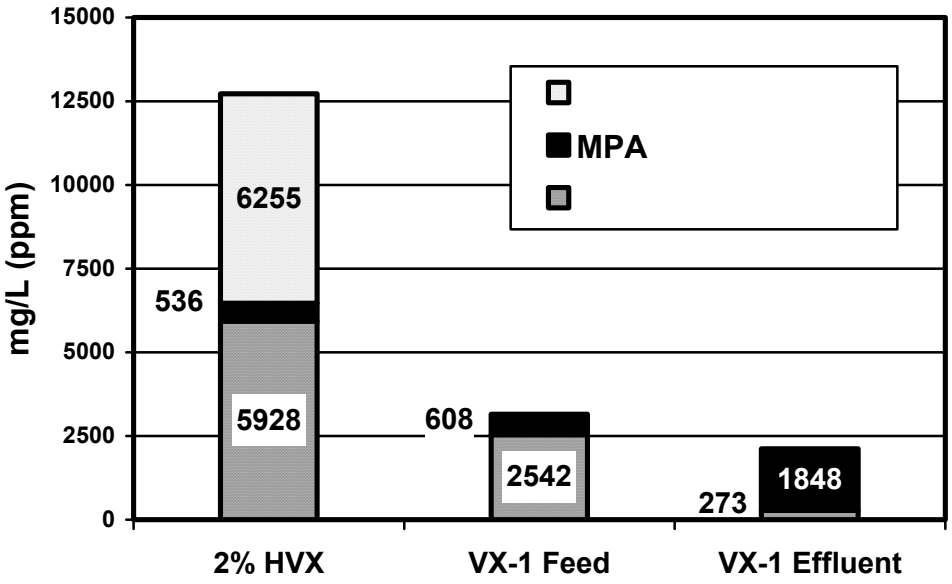

Figure 3. Degradation of CWC compounds by Method #2.

In line with the level of degradation, the elimination of toxicity was also not as thorough (Figure 4). However, an EC_{50} value of 50% represents very low toxicity in the Microtox® assay.

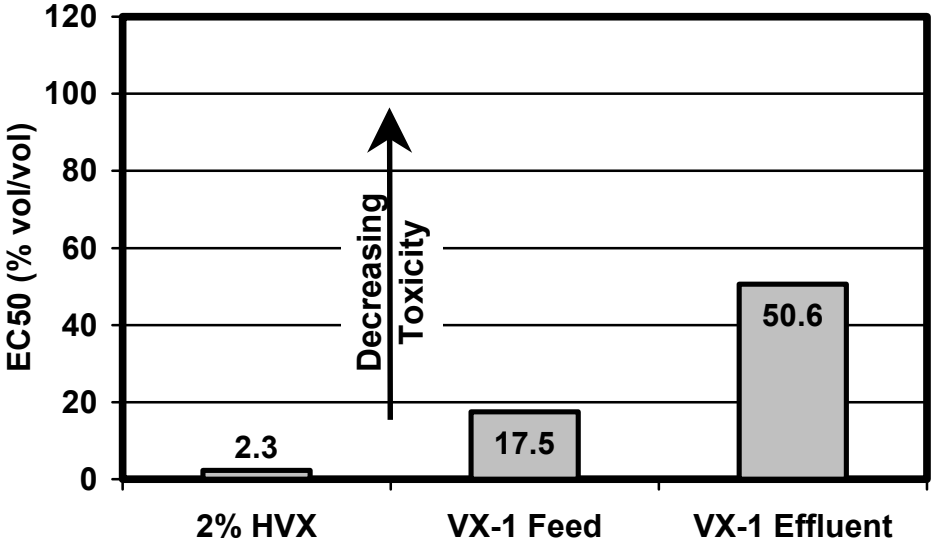

Figure 4. Microtox® results with feed and effluents from Method #2.

Method #3: The development of biomass within the column has been very slow getting started. More recently, the pretreatment was switched from ozone to peroxone (ozone plus hydrogen peroxide). In addition, small amounts of glucose and methanol have been added to the feed. This appears to have accelerated the production of biomass – in particular the *Methylobacterium radiotolerans* that can be readily identified by the bright pink patches of growth. Unfortunately, we do not have sufficient performance data yet to report in this paper. The information below in Figure 5 was obtained earlier, before the addition of extra carbon sources and the switch to peroxone.

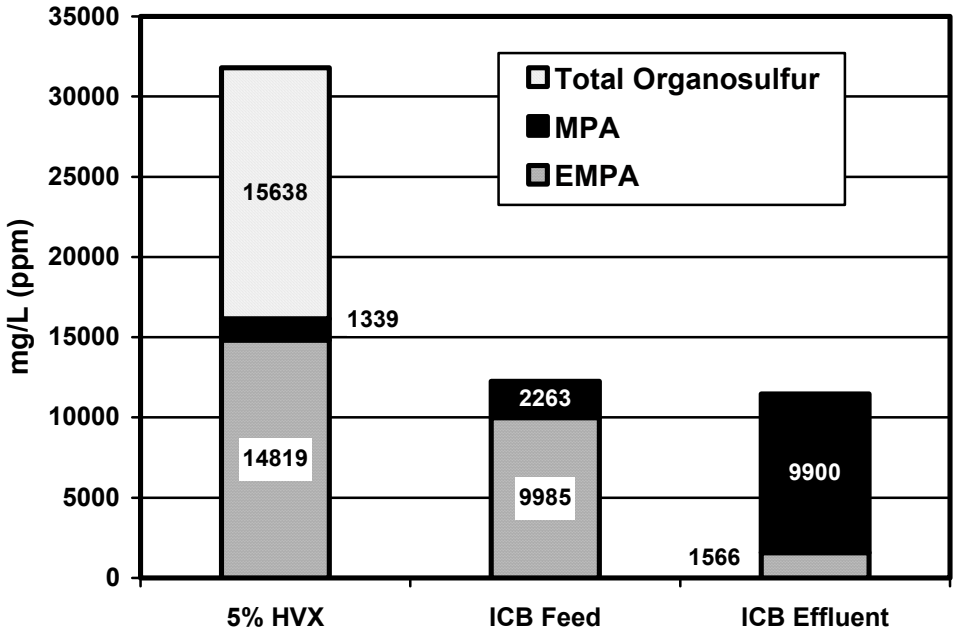

Figure 5. Degradation of CWC compounds by Method #3.

As with Method #2, the preoxidation step with ozone eliminates the organosulfur compounds and the bioreactor is able to convert the bulk of the EMPA to MPA.

CONCLUSIONS

At this stage of development, the Method #1 with two bioreactors and the intermediate oxidation step gave the best overall results in regards to removal of CWC compounds and reduction in toxicity. The disadvantage is that the initial bioreactor does not appear to be able to deal with high levels of HVX. Under the conditions used in this study, the maximum feed strength is ~2%. However, it is possible that modification of some of the parameters for the initial bioreactor (ex. addition of methanol to encourage proliferation of the *Methylobacterium radiotolerans,* lengthening the hydraulic residence time, etc.) will be able to extend its range.

Pretreatment with oxidation may be an effective means of providing a better substrate for biodegradation. For Method #2, modifications to the bioreactor parameters similar to those proposed above may result in a more complete removal of the MPA.

For Method #3, the initial results are promising, but it is still too early to conclude that it will be a viable approach or if peroxone gives a better feedstock material than ozone treatment. Use of other types of immobilized cell bioreactors, such as that developed by Allied Signal, Inc. [7,8] and others, may provide for more efficient feed distribution, pH control and aeration.

ACKNOWLEDGMENTS

We thank Mr. Mark Haley, Environmental Toxicology Team, U.S. Army Edgewood Chemical Biological Center, for conducting the Microtox® assays. This work was supported by the Assembled Chemical Weapons Assessment (ACWA) program, Program Manager for Chemical Demilitarization, Aberdeen Proving Ground, MD.

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